REMARKS

Rejections of Claims 1-3, 5-16, 20-26, and 31 Under 35 U.S.C. § 103(a)

Claims 1-3, 5-16, 20-26, and 31 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Sakai *et al.* (Sakai *et al.*, "Transplantation of Mesenchymal Stem Cells Embedded in Atelocollagen® Gel to the Intervertebral Disc: A Potential Therapeutic Model for Disc Degeneration," *Biomaterials*, 24: 3531-3541(2003)).

As noted in the previous responses to the prior Office Action, Sakai *et al.* do not teach or suggest administering uncultured mesenchymal stem cells (MSCs). Using a rabbit model, Sakai *et al.* disclose administering cultured MSCs for the treatment of intervertebral disc degeneration. Sakai *et al.* extracted bone marrow cells from the iliac crests and cultured the stem cells *ex vivo* for 12-15 days. During this cell culture expansion period, non-adherent cells (*i.e.*, non-mesenchymal stem cells) were removed every 48 hours to purify the adherent cells (*i.e.*, MSCs). After the cells were initially expanded, they were labeled with Ad-lacZ and embedded in Atelocollagen® gel matrix while the MSCs were further expanded in a cell culture medium (low-glucose Dulbecco's modified Eagle's medium (DMEM)) containing Atelocollagen® gel (Sakai *et al.*, page 3533, the bridging paragraph between the left and right columns). According to Sakai *et al* the MSCs embedded in Atelocollagen® gel-medium were transplanted into the nucleus pulposus of subject rabbits.

In response to Applicants' argument distinguishing the claimed invention from Sakai *et al.* and noting Sakai *et al.*'s culturing of the MSCs, the Examiner states that: "Sakai practices a more difficult method for the purposes of labeling the cells to confirm that they had survived in vivo" (December 28, 2007 Office Action, the bridging paragraph of pages 3-4). Applicants respectfully disagree with the Examiner's interpretation of Sakai *et al.* Sakai *et al.*, in fact, had another motivation to culture and purify the MSCs. Sakai *et al.* contemplated administering cultured MSCs from the outset, not only to label the MSCs to track their survival, but also to use Atelocollagen® gel as a carrier of the MSCs for transplantation. See the paragraph 12 of Declaration by Mohamed Attawia attached herewith (hereinafter, "Attawia Declaration"). Sakai *et al.* aimed to employ an Atelocollagen® gel matrix because the authors were aware of the advantages of using an Atelocollagen® gel matrix, and aimed to show that the matrix provides an

effective carrier. The title of Sakai *et al.*, "Transplantation of Mesenchymal Stem Cells Embedded in Atelocollagen® Gel to the Intervertebral Disc: A Potential Therapeutic Model for Disc Degeneration," indicates the authors' emphasis on the importance of using an Atelocollagen® gel matrix as a carrier in their experiment. Further, Sakai *et al.* recite in the Introduction: "Basic science research on culturing chondrocytes in Atelocollagen® gel has shown that cultures using Atelocollagen® gel result in greater matrix synthesis when compared to cells cultured in monolayer" (Sakai *et al.*, page 3532, 2nd paragraph). Sakai *et al.* embedded the MSCs into Atelocollagen® matrix by culturing the MSCs in low-glucose DMEM containing the liquid solution of Atelocollagen®. See paragraphs 13-14 of the Attawia Declaration. Thus, Sakai *et al.*'s emphasis on the use of the Atelocollagen® gel teaches away from use of uncultured cells in Applicants' claimed method. Therefore, Applicants respectfully submit that, based on Sakai *et al.*'s teaching, one of ordinary skill in the art would not have been motivated to practice Applicants' claimed invention (*i.e.*, administering uncultured MSCs to treat a degenerative spinal disc), but would have been highly motivated to culture and expand stem cells in order to embed the cells onto an Atelocollagen® gel matrix and to implement the purportedly advantageous Atelocollagen® gel matrix as a carrier.

Furthermore, administering uncultured mesenchymal stem cells would not have been obvious to a person having ordinary skill in the art (*i.e.*, stem cell regenerative medicine) because there was a low expectation of success. At the time of the invention, cell culture expansion was generally used not necessarily to label cells, but to expand and/or to induce differentiation of cells in stem cell regenerative medicine. It was a standard practice in the art to culture clinically useful cells (*i.e.*, mesenchymal stem cells) in order to obtain and maintain those useful cells at a higher number. Applicants previously provided a discussion of references published at the time of the invention, which all teach culturing clinically useful cells outside of the context of labeling (previous Amendment filed on June 12, 2007). Further, the Examiner states: "There would be a reasonable expectation of success because cells that are culture[d] for weeks, such as in Sakai *et al.*, are still viable and therapeutic. Therefore, newly isolated cells would be expected to work just as well, if not better" (the Office Action, page 7, the last paragraph). At the time of the invention, however, it was commonly known in the art that the MSCs available in the bone marrow of a donor for treatment were limited. See the Attawia Declaration, paragraphs 7 and

18. Further, Sakai *et al.* also teach that the MSCs are found in small numbers (Sakai *et al.*, page 3532, left column, 4th full paragraph). This statement is further supported by Centeno *et al.* (Centeno *et al.*, *Pain Physician*, 11(3): 343-353 (2008)) (attached as Exhibit A of the Attawia Declaration). As stated in paragraph 18 of the Attawia Declaration, Centeno *et al.* teach that the number of MSCs that can be isolated from bone marrow is limited: usually 1 out of 10,000-1,000,000 bone marrow nucleated cells (see Centeno *et al.* at page 345, right col., 1st paragraph). As a result, Centeno *et al.* note, "most research in cartilage regeneration has focused on the use of *culture expanded cells*" (*Id.* at page 345, right col. 2nd paragraph, *emphasis added*). Therefore, given the number of the MSCs available in bone marrow at the time, as well as the general knowledge available in the art regarding cartilage regeneration using cultured expanded cells, there was a low expectation of success to effectively treat a degenerated intervertebral disc without cell culture expansion. Due to this low expectation of success, it would not have been obvious to a person having ordinary skill in the art to practice the claimed subject matter.

Further, the Office Action states that Sakai *et al.* do not teach administration of a large number of cells because Sakai *et al.* administer 0.04 ml of cells at 1×10^6 cells/ml, *i.e.*, 40,000 cells per animal (Sakai *et al.*, page 3533; and the Office Action, page 4). Specifically, the Examiner states: "Sakai *et al.* are injecting a very small amount of cells probably comparable (absent evidence to the contrary) to the amount that would have been injected had Sakai *et al.* skipped the culture step" (the Office Action, page 4). Applicants respectfully disagree with the Examiner's analysis, because the cell number administered (*i.e.*, 40,000 mesenchymal stem cells purified and embedded in Sakai *et al.*'s Atelocollagen® gel) is still likely a much higher number than if Sakai *et al.* skipped the culture step. This is because the embedded stem cells in Sakai *et al.* are the adherent MSCs that were purified and expanded, in contrast to uncultured MSCs found among millions of other nucleated cells harvested (*see* Sakai *et al.* at page 3532, section 2.1, the last sentence; and Centeno *et al.* at page 345, right col. 1st paragraph). Centeno *et al.* describe purification of MSCs by removal of non-adherent cells during cell culture expansion, and note significantly more MSCs than if the harvested cells had not undergone cell culture expansion (*See* Centeno *et al.* at page 345, right col. second paragraph, last sentence). Without such cell culture expansion, one would require more bone marrow to obtain enough cells to administer 40,000 MSCs as described in Sakai *et al.* Therefore, one would have been highly

motivated to culture and expand the MSCs, especially if one desired to employ Atelocollagen® matrix as a carrier for transplant as in Sakai *et al.*

The Office Action states: "One of ordinary skill in the art would know that autologous cell administration has been a standard practice for bone-marrow transplants, blood transfusions, skin, and hair grafts long before the relevant priority date of 11/13/03" (the Office Action, page 4). Applicants respectfully submit that the Office Action is silent on whether or not these practices involve cell culture. The Office Action merely indicates that these practices employ "autologous" cells. Further, the Office Action is also silent on whether these practices specifically involve transplantation of the MSCs as recited in instant Claim 1, rather than other cell types such as red blood cells, platelets, white blood cells, skin cells or hair follicles. Thus, Applicants respectfully submit that it has not been established that these practices involve administration of "autologous uncultured mesenchymal stem cells" as claimed in the present application.

Further, the Office Action states: "One of ordinary skill would ch[o]ose to administer a volume appropriate for humans. In other words, it would be a matter of routine optimization to adapt the method of Sakai *et al.* to humans with appropriate volumes" (the Office Action, bridging paragraph between page 5 and 6). Applicants respectfully disagree with the Examiner. One of ordinary skill in the art would not have a motivation to optimize the administration volume because there is a low expectation of success in treating degenerative disc disease by administering the MSCs based on the teaching of Sakai *et al.* as discussed above. Even if one of ordinary skill in the art attempted to optimize the conditions, it would not have been obvious because the number of harvested mesenchymal stem cells in a human may be different from one patient to another, and the ratio of MSCs to other nucleated cells may differ as well, which may affect the final concentration and volume of cells required to be administered.

Applicants previously argued that the invention satisfied a long-felt need in the art. The contemporaneous references, including Sakai *et al.*, required culturing of cells as discussed above. The culturing process requires a delay in treating the patient's degenerating disc. The present invention allows a patient to undergo general anesthesia only once, under which both surgical procedures for removing bone marrow cells and for administering the MSCs to the disc can be performed. Further, it is well known in the art the conventional culturing method, for

example, one described in Sakai *et al.* is highly susceptible to deleterious impurities, contaminations, and exposure to chemical additives (see the Attawia Declaration, paragraphs 7-9). There was a long-felt, but unsatisfied, need to minimize the risk of unwanted contamination as well as to increase the clinical efficiency. Applicants submit that invention satisfied a long-felt need in the art by providing the claimed method of treating degenerative disc disease by administering autologous uncultured mesenchymal stem cells.

For the foregoing reasons, Applicants respectfully submit that instant Claim 1 and its dependency are not obvious in view of Sakai *et al.* Reconsideration and withdrawal of the rejection are requested.

Rejections of Claims 1-3, 6, 10-16, 31, and 33 Under 35 U.S.C. § 103(a)

Claims 1-3, 6, 10-16, 31 and 33 are rejected under 35 U.S.C. § 103(a) as being obvious over Sakai *et al.* as applied to Claims 1-3, 6-16, 20-26 and 31, and further in view of El-Khoury *et al.* (El-Khoury, G. *et al.*, "Percutaneous Procedures for the Diagnosis and Treatment of Lower Back Pain: Diskography, Facet-Joint Injection, and Epidural Injection," *Am. J. Roentgenol.*, 157(4): 685-691 (1991)).

Applicants' claims recite the use of uncultured autologous MSCs. Sakai *et al.* is discussed in detail above. According to the Examiner, El-Khoury *et al.* teach administration of an agent to an intervertebral disc at a total volume of 2.5 ml for the treatment of back pain (El-Khoury *et al.*, pages 686 and 688). Although El-Khoury *et al.* teach administration of steroids and anesthetic agents, they do not teach or suggest administration of uncultured autologous MSCs (page 687).

As discussed above and in the Attawia Declaration, the teachings of Sakai *et al.*, as well as common knowledge available in the art at the time of the invention, would not have motivated one of skill in the art to practice Applicants' claimed invention of administering uncultured autologous MSCs to treat degenerative disc disease. Sakai *et al.* do not teach or suggest using cultured cells, and, in effect, teach away from doing so, since they emphasize the value of using Atelocollagen® gel matrix as the MSC carrier, and teach culturing the cells for use with that gel.

Further, one of skill in the art reading both references would not have combined them to arrive at the claimed invention. As discussed in the previous section, there would have been a

low expectation of success for skipping the culture expansion step in Sakai *et al.*, and for arriving at Applicants' invention.

Therefore, the combination of Sakai *et al.* and El-Khoury *et al.* do not render Claims 1-3, 6, 10-14, 16, 31 and 34 obvious for the use of uncultured MSCs. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejections of Claims 1-3, 6, 10-14, 16, 31, and 34 Under 35 U.S.C. § 103(a)

Claims 1-3, 6, 10-14, 16, 31, and 34 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Sakai *et al.* as applied to Claims 1-3, 6-16, 20-26 and 31 and further in view of McMillan *et al.* (McMillan, D. *et al.*, "Intra-Operative Autologous Blood Management," *Transfusion and Apheresis Science*, 27(1): 73-81 (2002)).

Applicants' claims recite the use of uncultured autologous MSCs. Sakai *et al.*, discussed in detail above, teach the use of cultured MSCs, and, in fact, teach away from the use of autologous uncultured MSCs. According to the Examiner, McMillan *et al.* teach intraoperative autologous blood cell administration in which the autologous blood cells are re-transfused into a patient after harvesting from the same patient. The Office Action states: "It would be obvious to one of ordinary skill in the art to combine the mesenchymal stem cell treatment of Sakai *et al.* with the intraoperative procedure of McMillan et al. because one could treat degenerative discs immediately and without the problem of rejection."

Applicants respectfully disagree. At the time of the invention, one of skill in the art would not have been motivated to combine the cited references to arrive at Applicants' claimed invention. McMillan *et al.*'s teachings are directed to blood transfusion for peri- and intra-operative surgical procedures, such as cardiac surgery. Thus, this teaching is in a non-analogous art, and would not be in the field of research of one skilled in the art of degenerative disc disease. Further, one of skill in the art would have understood that the red blood cells described in McMillan, *et al*, are far more plentiful in the human body than mesenchymal stem cells, which exist in the body in small numbers. As noted above, Sakai *et al.* teach that the MSCs are found in small numbers (Sakai *et al.*, page 3532, left column, 4th full paragraph). Thus, it would not be expected that mesenchymal stem cells could be substituted for the blood cells in McMillan *et al.*'s method.

Therefore, the combination of Sakai *et al.* and McMillan *et al.* do not render Claims 1-3, 6, 10-14, 16, 31 and 34 obvious for the use of uncultured MSCs. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejections of Claims 1-4, 6, 11-16, 20-24, 31 and 33 Under 35 U.S.C. § 103(a)

Claims 1-4, 6, 11-16, 20-24, 31 and 33 have been rejected under 35 U.S.C. § 103(a) as being unpatentable over Sakai *et al.* as applied to Claims 1-3, 6-16, 20-26 and 31, and further in view of Tanny *et al.* (Tanny, G.B. *et al.*, "Improved Filtration Technique for Concentrating and Harvesting Bacteria," *Appl. Environ. Microbiol.*, *40*(2):269-273 (1980)).

Applicants respectfully disagree. At the time of the invention, one of skill in the art would not have been motivated to combine the cited references to arrive at Applicants' claimed invention. Applicants' claims recite the use of uncultured autologous MSCs. Sakai *et al.*, discussed in detail above, teach the use of cultured MSCs, and, in fact, teach away from the use of autologous uncultured MSCs. Tanny *et al.* merely teach concentrating cells by filtration and harvesting bacterial cultures. It should be noted that, similar to Sakai *et al.*, the bacterial cells discussed in Tanny *et al.* were also cultured. Moreover, this teaching is a non-analogous art, and would not be in the field of research of one skilled in the art of degenerative disc disease. Therefore, Tanny *et al.* also support Applicants' argument that culturing was a standard practice at the time of the invention.

Thus, Sakai *et al.* and Tanny *et al.*, in combination, would not have motivated one of ordinary skill in the art to administer uncultured MSCs into a degenerated spinal disc.

Therefore, one of ordinary skill in the art would not be motivated to combine the teachings of Sakai *et al.* with Tanny *et al.*, based on these references or the knowledge of one of ordinary skill in the art, with any reasonable expectation of success, to arrive at the claimed invention. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejections of Claims 1-3, 5-7, 10-16, 18, 20-24, and 31-32 Under 35 U.S.C. §103(a)

Claims 1-3, 5-7, 10-16, 18, 20-24, and 31-32 have been rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Sakai *et al.* as applied to Claims 1-3, 6, 11-16, 20-24, 31, and 33

above, in further in view of Russell *et al.* "Human Bone Marrow Mesenchymal Stromal Cells as a Source of Chondrocytes for Treatment of Intervertebral Disc Degeneration," 27, *Abstracts of the 30th Annual Meeting of the International Society for the Study of the Lumbar Spine*, Vancouver, Canada (May 2003).

None of the references, in combination, teaches or suggests administering autologous uncultured mesenchymal stem cells into a degenerated intervertebral disc. Sakai *et al.*, discussed in detail above and in the Attawia Declaration, teach the use of cultured MSCs, and, in fact, teach away from the use of autologous uncultured MSCs. As discussed above, one of skill in the art would not have had been motivated to use uncultured MSCs for treatment of degenerative disc disease. Russell *et al.* teach the use of human bone marrow mesenchymal stromal cells as a source of chondrocytes for the treatment of intervertebral disc degeneration. Significantly, Russell *et al.* also teach that the cells were cultured in the presence of a growth factor, TGF-β1. Thus, one of ordinary skill in the art would not be motivated to combine the teachings of Sakai *et al.* with Russell *et al.* to arrive at Applicants' invention of administering autologous uncultured mesenchymal stem cells into a degenerated intervertebral disc.

Further, as indicated above, one of skill in the art would not have been motivated to combine the teachings of the references to optimize the volume with a reasonable expectation of success.

Therefore, one of ordinary skill in the art would not be motivated to combine the teachings of Sakai *et al.* with Russell *et al.*, based on these references or the knowledge of one of ordinary skill in the art, with any reasonable expectation of success, to arrive at the claimed invention. Reconsideration and withdrawal of the rejection are respectfully requested.

Rejections of Claims 1-3, 6, 10-16, 31 and 33 Under 35 U.S.C. § 103(a)

Claims 1-3, 6, 10-16, 31 and 33 have been rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Sakai *et al.* as applied to Claims 1-3, 6, 11-16, 20-24, 31, and 33 above, in further in view of Russell *et al.* "Human Bone Marrow Mesenchymal Stromal Cells as a Source of Chondrocytes for Treatment of Intervertebral Disc Degeneration," 27, Abstracts of the 30th Annual Meeting of the International Society for the Study of the Lumbar Spine, Vancouver, Canada (May 2003), and in further in view of El-Khoury *et al.* (El-Khoury, G. *et al*,

"Percutaneous Procedures for the Diagnosis and Treatment of Lower Back Pain: Diskography, Facet-Joint Injection, and Epidural Injection," *Am. J. Roentgenol* , 157(4): 685-691 (1991)).

Sakai *et al.*, discussed in detail above and in the Attawia Declaration, teach the use of cultured MSCs, and, in fact, teach away from the use of autologous uncultured MSCs. As discussed above, one of skill in the art would not have had been motivated to use uncultured MSCs for treatment of degenerative disc disease. However, Sakai *et al.* do not teach administering TGF-β in conjunction with the uncultured MSCs.

The Russell *et al.* reference is discussed in detail above. Specifically, Russell *et al.* teach that the mesenchymal stem cells were cultured in the presence of a growth factor, TGF-β1. However, Russell *et al.* do not teach administration of uncultured MSCs.

The El-Khoury *et al.* reference is discussed in detail above. Although El-Khoury *et al.* teach administration of steroids and anesthetic agents, they do not teach or suggest administration of MSCs, or administration of any other uncultured cells (page 687).

The combination of these references, combined with what was known in the art, does not teach or suggest administration of the uncultured MSCs. Therefore, one of ordinary skill in the art would not be motivated to combine the teachings of Sakai *et al.* with Russell *et al.* and El-Khoury *et al.* based on common knowledge at the time of the invention. Therefore, the invention is not obvious. Reconsideration and withdrawal of the rejection are respectfully requested.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By _____

Deirdre E. Sanders
Registration No. 42,122
Telephone: (978) 341-0036
Facsimile: (978) 341-0136

Concord, MA 01742-9133

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